

In re: Application of STEINDLER, et al.

Confirmation No: 6329

Application No.: 10/695,600

Examiner: SAJJADI, F. G.

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REMARKS

Claims 30-39 were pending in the application at the time the Office Action was mailed. Claims 30-39 are rejected. No claims were allowed.

Claim 30 has been amended to recite “an *ex vivo* population” of cells. Support for this amendment is found throughout the specification. See, for example page 6, lines 5-23 which describes the isolation technique; Figure 1 which is a microscopic view of type I-III clones. No new matter has been added by virtue of this amendment and entry is respectfully requested. These amendments were made solely for purposes of expediting prosecution and are not meant to be construed as surrender of any subject matter. Applicants reserve the right to further prosecute the subject matter in one or more Divisional or Continuation applications.

Claim Rejections Under 35 U.S.C. § 112, Written Description

Claims 30-39 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

Applicants respectfully traverse.

The Examiner asserts that “the instant specification only demonstrates possession of a mixed population of human or mouse brain progenitor cells, and further, Applicants have failed to demonstrate possession of any isolated cell types, given that the mixed culture of brain multipotent progenitor cells represent a continuum of cell proliferation and differentiation, wherein different cell types are simultaneously immunonegative for said markers. As such, no population of cells may be demonstrated as isolated.”

Applicants respectfully disagree. For example, Applicants have described the stem cells and identified the actual markers that identify these stem cells, i.e. Type I, II and II cells.

Applicants also provide detailed morphological and phenotypic descriptions. For example, page 5, lines 11-31 describe the phenotypic and morphological data:

Figure 1 shows phase contrast and electron microscopic images of type I, II, and III clones. Figures 1A, 1C, and 1E are phase contrast images of type I, II and III clones of cultured adult brain cells, respectively. Figures 1B, 1D, and 1F show type I, II and III spheres counterstained with propidium iodide, respectively. Scale bars for Figures 1A-F are 40, 30, 20, 30, 20, and 30 microns, respectively;

Figure 2 depicts the types of spheres found in the culture paradigm of the invention, and the generation conditions for the appearance and evolution of sphere types from brain;

Figure 3 shows the phase and electron microscopic images of type II (A and B) and type III (C and D) spheres. Scale bars for Figures 3A-D are 10, 5, 15, and 2 microns, respectively;

Figure 4 shows immunostaining of early and late type II and type III spheres. Scale bars for Figures 4A, G and J are 10 microns, Figures 4B and 4C are 15 microns, Figures 4E and 4F are 30 microns, Figure 4H is 20 microns, and Figure 4I is 100 microns;

Figure 5 shows the evolution and proliferation of type II (Figure 5A and 5C), and type III (Figures 5B and 5D) spheres. Scale bars for Figures 5A and 5B are 25 microns, and for Figures 5C and 5D are 10 microns;

Figure 6 shows type II and type III spheres from ROSA-26 transgenic mice. Scale bars for Figure 6A is 50 microns, and Figure 6B is 30 microns; and

Figure 7 shows phase and electron microscopy of a type II adult mouse and type II adult human sphere. The adult mouse sphere is approximately 100 microns in diameter, while the adult human sphere is approximately 200 microns in diameter.

Applicants describe the isolation of these cells, see, for example page 6, lines 5-23:

Cell separation and cell adhesion can be manipulated using a variety of contact-limiting and contact-inhibiting factors. For example, chemical-separating agents such as mercaptoethanol, physical separating agents such as methylcellulose, and anti-adhesives such as poly-2-hydroxyethyl methacrylate are used to deter cell-cell and cell-substrate associates during the initial isolation of stem/precursor cells from the newly-dissociated brain. This allows the “purification” of these cells from

mature, differentiated neurons and glia that are also dissociated during the brain dissociation procedures. The mature, differentiated neurons and glia cannot survive these anti-adhesion, anti-cell interaction procedures. Thus, agents such as mercaptoethanol are always used in the first stage of isolation of type I and II clones to help deter the survival of the more mature cellular elements (by deterring their clustering). At the same time, agents such as mercaptoethanol may have certain growth-promoting actions on the single stem/precursor cells that eventually proliferate to form these early sphere types.

Since cell-cell and cell-substrate interactions are important for cellular differentiation, contact-inhibiting (or contact-limiting) factors as mercaptoethanol are eventually removed from the culture medium for the evolution or differentiation of type II and type III spheres.

The differentiation of type m spheres requires other additional factors, including growth factors like beta fibroblast growth factor, epidermal growth factor, or such factors that are also contained within pituitary extract. Such additional factors are described in the type III culture media discussed below (see, Example 3).

Further Applicants describe the types of purified stem cells. See, for example, page 7, lines 16-33 through to page 12, lines 1-6. See, also Examples 1-3 beginning on page 14 through to page 16, lines 1-5. However, in order to expedite and compact prosecution, Applicants have amended claim 30 to recite an *ex vivo* culture of a population of multipotent, progenitor or precursor brain stem cells that are immunonegative for glial fibrillary protein, nestin and TuJ1 when cultured under conditions that prevent or reduce cell-cell and cell-substrate interactions, wherein the brain stem cells are from a mammal selected from the group consisting of: human and mouse. Claims 31-39 depend from claim 30 and as such encompass all the limitations of the independent claim 30. These amendments were made solely for purposes of expediting prosecution and are not meant to be construed as surrender of any subject matter. Applicants reserve the right to further prosecute the subject matter in one or more Divisional or Continuation applications.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejections Under 35 U.S.C. § 112, Enablement

Claims 30-39 stand rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. Claim 30 as amended herein recites an *ex vivo* culture of a population of multipotent, progenitor or precursor brain stem cells that are immunonegative for glial fibrillary protein, nestin and TuJ1 when cultured under conditions that prevent or reduce cell-cell and cell-substrate interactions, wherein the brain stem cells are from a mammal selected from the group consisting of: human and mouse. Claims 31-39 depend from claim 30 and as such encompass all the limitations of the independent claim 30.

Applicants reiterate:

For example, Applicants have described the stem cells and identified the actual markers that identify these stem cells, i.e. Type I, II and III cells. Applicants also provide detailed morphological and phenotypic descriptions. For example, page 5, lines 11-31 describe the phenotypic and morphological data:

Figure 1 shows phase contrast and electron microscopic images of type I, II, and III clones. Figures 1A, 1C, and 1E are phase contrast images of type I, II and III clones of cultured adult brain cells, respectively. Figures 1B, 1D, and 1F show type I, II and III spheres counterstained with propidium iodide, respectively. Scale bars for Figures 1A-F are 40, 30, 20, 30, 20, and 30 microns, respectively;

Figure 2 depicts the types of spheres found in the culture paradigm of the invention, and the generation conditions for the appearance and evolution of sphere types from brain;

Figure 3 shows the phase and electron microscopic images of type II (A and B) and type III (C and D) spheres. Scale bars for Figures 3A-D are 10, 5, 15, and 2 microns, respectively;

Figure 4 shows immunostaining of early and late type II and type III spheres. Scale bars for Figures 4A, G and J are 10 microns, Figures 4B and 4C are 15 microns, Figures 4E and 4F are 30 microns, Figure 4H is 20 microns, and Figure 4I is 100 microns;

Figure 5 shows the evolution and proliferation of type II (Figure 5A and 5C), and type III (Figures 5B and 5D) spheres. Scale bars for Figures 5A and 5B are 25 microns, and for Figures 5C and 5D are 10 microns;

Figure 6 shows type II and type III spheres from ROSA-26 transgenic mice. Scale bars for Figure 6A is 50 microns, and Figure 6B is 30 microns; and

Figure 7 shows phase and electron microscopy of a type II adult mouse and type II adult human sphere. The adult mouse sphere is approximately 100 microns in diameter, while the adult human sphere is approximately 200 microns in diameter.

Applicants describe the isolation of these cells, see, for example page 6, lines 5-23:

Cell separation and cell adhesion can be manipulated using a variety of contact-limiting and contact-inhibiting factors. For example, chemical-separating agents such as mercaptoethanol, physical separating agents such as methylcellulose, and anti-adhesives such as poly 2-hydroxyethyl methacrylate are used to deter cell-cell and cell-substrate associates during the initial isolation of stem/precursor cells from the newly-dissociated brain. This allows the "purification" of these cells from mature, differentiated neurons and glia that are also dissociated during the brain dissociation procedures. The mature, differentiated neurons and glia cannot survive these anti-adhesion, anti-cell interaction procedures. Thus, agents such as mercaptoethanol are always used in the first stage of isolation of type I and II clones to help deter the survival of the more mature cellular elements (by deterring their clustering). At the same time, agents such as mercaptoethanol may have certain growth-promoting actions on the single stem/precursor cells that eventually proliferate to form these early sphere types.

Since cell-cell and cell-substrate interactions are important for cellular differentiation, contact-inhibiting (or contact-limiting) factors as mercaptoethanol are eventually removed from the culture medium for the evolution or differentiation of type II and type III spheres.

The differentiation of type m spheres requires other additional factors, including growth factors like beta fibroblast growth factor, epidermal growth factor, or such factors that are also contained within pituitary extract. Such additional factors are described in the type III culture media discussed below (see, Example 3).

Further Applicants describe the types of purified stem cells. See, for example, page 7, lines 16-33 through to page 12, lines 1-6. See, also Examples 1-3 beginning on page 14 through to page 16, lines 1-5. In addition, MPEP 2164.08 states that "[a]ll that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill

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in the art. Further the scope of enablement must only bear a 'reasonable correlation' to the scope of the claims. See, e.g., *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). See MPEP 2164.01 which cites *United States v. Teletronics, Inc.*, 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988) ("The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with the information known in the art without undue experimentation."). Applicant asserts that because of the high level of skill in the art and the state of the art at the time the application was filed, one of ordinary skill in the art would not have to perform undue experimentation to make and use the invention as claimed.

Applicants submit that the amended claims now overcome the Examiner's rejections. The specification does describe the isolation and culturing of a population of multipotent, progenitor or precursor brain stem cells that are immunonegative for glial fibrillary protein, nestin and TuJ1 when cultured under conditions that prevent or reduce cell-cell and cell-substrate interactions, wherein the brain stem cells are from a mammal selected from the group consisting of: human and mouse as recited in claim 1. See line 25, page 3 through line 1, page 4, lines 5-23, page 6, and Examples 3 and 10. Dependent claims 31, 32, and 35-37 depend on independent claim 30 and as such encompass all of the claim limitations of claim 30. These amendments were made solely for purposes of expediting prosecution and are not meant to be construed as surrender of any subject matter. Applicants do not necessarily agree with or acquiesce in these rejections. Applicants reserve the right to further prosecute the subject matter in one or more Divisional or Continuation applications.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

CONCLUSION

Applicants have made every effort to present claims which overcome the Examiner's assertions, and it is believed that all claims are now in condition for allowance. However,

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Applicants request that the Examiner call the undersigned (direct line 561-671-3623) if anything further is required by the Examiner prior to issuance of a Notice of Allowance for all claims.

Applicants respectfully request entry of the foregoing amendments and remarks and reconsideration and withdrawal of all rejections. It is respectfully submitted that this application with claims 30-32 and 35-37 is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for any retroactive extensions of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

Respectfully submitted,

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